ABSTRACT

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Title of Diploma Thesis: Comparison of separation using different types of stacionary phases

The goal of this diploma thesis was to compare two different stationary phases's efficiency of separation using HPLC method. Tested columns were a column filled with fused-core particles Ascentis Express C18 (Supelco Analytical) and a monolithic column Chromolith High Resolution RP-18 endcapped (Merck). As a mobile phase a mixture of acetonitrile and water was used and as a model sample a mixture of α-estradiol, β-estradiol, estrone, estriol, ethinylestradiol and ethylparaben as an internal standard was tested. Firstly modifications of the conditions of the separation process were discovered and optimal conditions of the separation for each analytical column were compared. According to them a conclusion of the efficiency of the columns was made. Based on the obtained results it was discovered that better results were achieved using the Chromolith High Resolution column, on which chromatograms with more resolved peaks and generating a lower back pressure than the Ascentis Express C18 column were found and so shorter analysis time using higher flow rate of the mobile phase can be achieved.